

Trisethylene-Imino-s-Triazine (Triethylene Melamine or TEM) in the Treatment of Neoplastic Diseases

MICHAEL B. SHIMKIN, M.D., HOWARD R. BIERMAN, M.D., KEITH H. KELLY, M.D.,
ELIZABETH LOWENHAUPT, M.D., ARTHUR FURST, Ph.D., *San Francisco*

SUMMARY

Trisethylene-imino-s-triazine (triethylene melamine or TEM) produced minimal effects in inhibiting transplantable lymphoma and mammary adenocarcinoma in mice. In strain A mice, injection of the compound induced pulmonary tumors.

TEM was tried on 32 patients with neoplastic disease, including nine patients with Hodgkin's disease and five with lymphosarcoma and lymphatic leukemia. The therapeutic and toxic effects were similar to those observed with nitrogen mustard (HN2). Satisfactory remissions of up to three months were observed in Hodgkin's disease and lymphosarcoma following parenteral administration of TEM. It is the authors' impression that the remissions obtained with TEM were not as complete and did not last as long as those obtained with HN2.

TEM is effective by the oral route as well as parenterally, and produces much less emetic reaction than HN2. On the other hand, the chemotherapeutic range is narrower than that of HN2. Patients who do not respond to HN2 show no response to TEM.

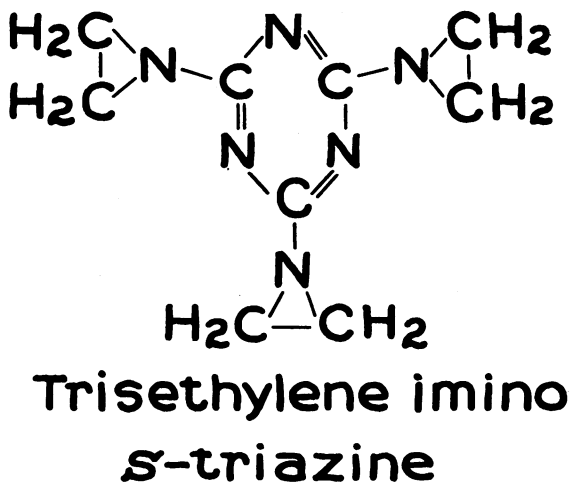
TEM is a drug of some clinical usefulness in the same conditions and with the same general limitations and toxic effects as HN2. The ease of administration of TEM increases its hazards, and close clinical and hematologic observations are essential on patients receiving the agent.

THE discovery that certain nitrogen mustard compounds have ameliorative effects in Hodgkin's disease and related lymphomas⁴ has led to the synthesis and biologic testing of a wide spectrum of related chemicals for their tumor-inhibiting properties. The investigations thus far have not uncovered agents which by clinical standards are clearly supe-

From the Laboratory of Experimental Oncology, National Cancer Institute, National Institutes of Health, Public Health Service, Federal Security Administration and the Division of Medicine, University of California School of Medicine, San Francisco (Shimkin, Bierman, Kelly); the Division of Pathology, University of California School of Medicine, San Francisco (Lowenhaupt); and the Division of Chemistry, University of San Francisco, San Francisco (Furst).

The work reported was supported in part by Research Grant C-396 from the Division of Research Grants and Fellowships of the National Cancer Institute, Public Health Service. Dr. Kelly is a Damon Runyon Fellow.

Chart 1.—Chemical structures of TEM.



rior to methyl-*bis* (B-chloroethyl) amine (HN2) or methyl-*tris* (B-chloroethyl) amine (HN3). These powerful vesicants must be given directly into the blood stream, and they produce severe nausea and vomiting as well as occasional phlebothrombosis.

A number of compounds related to the nitrogen mustards have been devised recently in England^{7, 9} and the United States^{3, 6} which appear to have effects similar to the nitrogen mustards but which have less emetic reaction and are effective when administered by the oral route. Among these compounds (Chart 1) is trisethylene-imino-*s*-triazine.

The present report deals with the laboratory and clinical investigations of trisethylene-imino-*s*-triazine.* This compound is more familiarly known as triethylene melamine or TEM, as it will be referred to hereinafter.

ANIMAL STUDIES

Toxic effects. Mice of strains A and C3H, three months old and weighing approximately 25 gm., tolerated doses of 0.04 mg. of TEM dissolved in water or saline solution and injected intraperitoneally or intravenously. Approximately half of the group of animals died within five days following doses of 0.1 mg. There was a precipitous drop in the number of circulating leukocytes, and a moderate drop in the number of platelets within four days following

*The compound was made available for this study in November 1949 by Dr. Frank L. Rose of the Imperial Chemical Industries, Manchester, England. Dr. M. L. Crossley of the American Cyanamide Company supplied tablets of the material for oral use.

the administration of 0.1 mg. In histological examination of the tissues of the mice, the following phenomena were observed: Vascular engorgement of the viscera, disappearance of lymphocytes with atrophy of Malpighian corpuscles in the spleen, loss of cells from the follicles of lymph nodes, pronounced decrease in the cellular elements of both series in the bone marrow, and necrosis of the germinal epithelium of the testes.

Effect on tumors. The effects of TEM on tumor growth were investigated in strain A mice implanted with a transplantable lymphoma, and in strain C3H mice bearing a transplanted mammary adenocarcinoma. The animals were given 11 daily intraperitoneal injections of 0.0025 or 0.005 mg. of TEM dissolved in water, for a total dose of 0.0325 mg. Six mice were sacrificed at 12 days and the weights of the tumors and of the carcasses were compared with those of six untreated animals. The lymphomas in mice treated with TEM weighed an average of 1.45 gm. (0.9 gm. to 2.1 gm.), whereas the untreated tumors weighed 3.2 gm. (2.7 gm. to 4.6 gm.). The effect on mammary tumors was much less pronounced; the tumors of the treated animals weighed 2.5 gm. on the average, whereas the untreated tumors weighed 2.9 gm. The total body weight of the animals was not affected.

The experiment was repeated with ten mice in the treated and untreated groups of each strain. The strain A mice with the lymphoma treated with TEM lived for an average of 36 days (29 to 38) whereas the controls died at an average of 33 days (29 to 37); the tumor obviously grew rapidly after the conclusion of the treatment. The strain C3H mice with mammary tumors treated with TEM lived an average of 31 days (22 to 51) whereas the untreated controls survived for 23 days (12 to 35). In all animals there was progressive growth of the neoplasm even while under active treatment with TEM, and all the animals died of the neoplastic growth.

Reported effects on other transplantable lymphomas, sarcomas and carcinomas in the mouse^{3, 6} and on the Walker rat carcinoma⁹ indicate more pronounced inhibition of tumor growth than was elicited in the present investigation.

Carcinogenic effect. It has been pointed out¹¹ that many chemical agents which have inhibitory effects on tumor growth also have carcinogenic properties. The nitrogen mustards, for example, induce pulmonary tumors in mice.⁵

TEM was administered intravenously to 20 strain A mice two months of age. The initial dose of 0.05 mg. in 0.1 cc. of saline solution was followed by two doses of 0.025 mg. at monthly intervals, for a total dose of 0.1 mg. Six additional mice received single intraperitoneal injections of 0.05 mg. Fifteen mice were maintained as untreated controls. The mice were sacrificed 18 weeks following the initial injection, and the lungs were examined for the presence of pulmonary tumors.

Of the 15 untreated controls, two had solitary pulmonary tumors. This incidence coincides with the

10 to 15 per cent incidence of spontaneous pulmonary tumors in strain A mice six to seven months of age.¹⁰

The six mice that received 0.05 mg. of TEM intraperitoneally had 2, 3, 0, 2, 1, and 0 pulmonary tumors, respectively, or an average of 1.3 tumors per animal. The 20 mice injected with 0.1 mg. intravenously had an average of 2.1 pulmonary tumors per animal; four mice had no tumors, four had single tumors, eight had two to three tumors, and four had four to six nodules in the lungs.

Four C3H male mice injected subcutaneously or intraperitoneally with 0.05 mg. of TEM were killed one year later. No tumors at the site of injection were found, but two mice had one and two pulmonary tumors, respectively.

It is concluded that TEM is carcinogenic for the pulmonary tissue of mice, and that its carcinogenic potency is of the same order as found for HN2 by Heston.⁵

CLINICAL STUDIES

Since February 1950, 32 patients with advanced neoplastic disease have received 60 therapeutic courses of TEM. Of the patients, 30 were treated at the Laboratory and two at Letterman Army Hospital.* The diagnoses in all cases were based on microscopic examination of at least one relevant biopsy specimen.

TEM is a white powder that is immediately soluble in water or in saline solution. Occasional samples of TEM contain granules or flakes which do not dissolve, indicating that polymerization has occurred and that the material should not be used. The compound is relatively stable in solution, and can be used for at least 24 hours if maintained in the refrigerator. For intravenous or intramuscular injection TEM is dissolved in sterile physiological saline, in concentrations of 1 or 2 mg. per cc.

For oral use, tablets of 5 mg. in a bland binder were employed. These were given with water, usually a half-hour before the noon meal.

Information regarding the patients and the treatment with TEM is given in Tables 1 and 2. There was no difference in toxic or therapeutic effects when the agent was given intramuscularly or intravenously, nor when the total dose was given in a single injection or in daily injections for three to seven days. The therapeutic dose or single course of TEM, if given intravenously or intramuscularly, is approximately 0.15 mg. per kilogram of body weight and should not exceed 0.25 mg. per kilogram of body weight. The agent should not be readministered until the hematologic status of the patient has returned to normal, which usually occurs within four to five weeks.

One patient (Case 19) with disseminated epidermoid carcinoma, primary in the nasopharynx, was injected intravenously with 0.5 mg. per kilogram of body weight and died on the 14th day showing hemorrhagic diathesis associated with pancytopenia.

*The staff of Letterman Army Hospital granted permission to use records on these two patients.

With oral medication, daily doses of 5 to 10 mg. for a total of 0.4 mg. per kilogram of body weight appear to be the safe upper limit for a single course of treatment. One patient (Case 4) with Hodgkin's disease received 0.9 mg. per kilogram of body weight during a course of two weeks, and hemorrhagic diathesis with petechiae, thrombopenia, severe leukopenia, and anemia developed. Repeated transfusions of whole blood, antibiotics, and other supportive measures were required.

Patients with lymphocytic leukemia appear to be extremely sensitive to TEM, and an initial parenteral dose of 0.05 mg. per kilogram of body weight, or 5 mg. orally, should not be exceeded until the effects of these lower doses are well established on the individual patient.

PHARMACOLOGIC EFFECTS

Immediate effects. In three cases study was made of electrocardiographic tracings, blood pressure, pulse and respirations during and for several hours following the intravenous administration of 0.1 mg. per kilogram of body weight. There were no significant changes. There were no general or local reactions to the agent within the first hour with any of the three routes of administration employed.

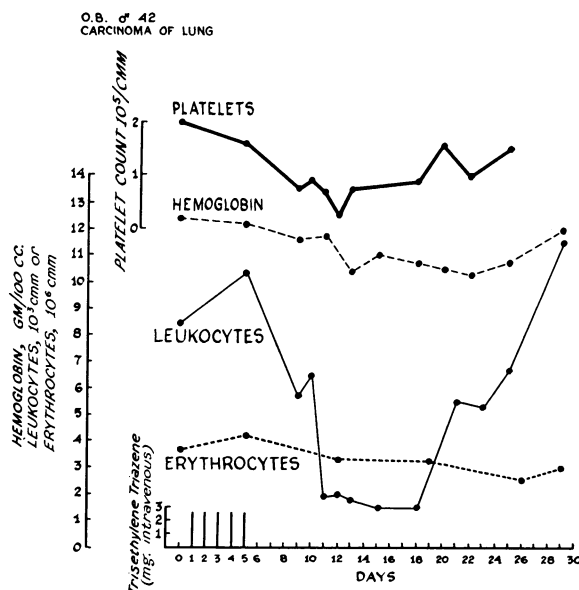
Local effects. In all but one patient, no serious local reactions occurred at the site of intravenous or intramuscular injection. Approximately one-third of the patients complained of some soreness at the site of intramuscular injection. In one patient (Case 15) with myelocytic leukemia, pronounced induration and inflammation occurred in the sites of intramuscular injection, and intravenous injection produced tenderness and redness along the course of the vein.

Gastrointestinal effects. Patients receiving over 0.2 of TEM per kilogram of body weight by intramuscular or intravenous routes experienced nausea for two to four days after completion of the course. Approximately one-third of these patients had one to four episodes of vomiting. The reaction was much less pronounced than that with HN2, and was usually delayed for four to six hours, as compared with the reaction to HN2, which usually occurs within an hour. With parenteral doses of 0.05 mg. per kilogram of body weight, or by oral route of administration, only mild nausea was encountered, unless the agent in a concentrated solution was injected rapidly into the vein. Diarrhea following treatment with TEM was noted in two patients receiving large doses of the agent.

Severe frontal headache, observed occasionally following HN2, was not observed in the 32 patients of this series treated with TEM.

Hematologic effects. The chief toxic effect of TEM is on the hematopoietic system. This effect is in every way comparable to the effects observed with HN2. Chart 2 illustrates the characteristic alterations observed on the peripheral blood picture of a patient with a bronchogenic carcinoma who received a dose of 0.25 mg. per kilogram of body weight.

Chart 2.—Characteristic effect of TEM on the cell count in peripheral blood. The patient with bronchogenic carcinoma (Case 21) received an intravenous course of 0.25 mg. per kilogram of body weight.



Within ten days there was a rapid decrease in the leukocyte and the platelet count, and a slower decrease in the erythrocyte count and hemoglobin value. In bone marrow aspirations at this time pronounced diminution in hematopoiesis, both of the white and red cell elements, was noted. Recovery from this depression began approximately three weeks after the drug was given.

With oral administrations, the hematologic depression was somewhat slower, particularly if the drug was administered at intervals of two or three days. The nadir of the leukocyte depression was usually observed in approximately two weeks and the count returned to normal within six weeks.

Other effects. In study of clinical and laboratory determinations before treatment with TEM and for as long as three months following treatment, no other major toxic effects that could be attributed to TEM were noted. In repeated examinations of the urine no significant changes were observed. Liver function, as measured by blood bilirubin, thymol turbidity, bromsulfalein retention, and cholesterol fractions, was not definitely changed. In one patient with Hodgkin's disease with jaundice, icterus was reduced following TEM therapy. Serum non-protein nitrogen, serum proteins, sodium, potassium and chloride were determined before and after treatment. There was no change in these factors. The uric acid level of the blood may be elevated for approximately one week following large doses of TEM.

THERAPEUTIC EFFECTS

Hodgkin's disease. All nine patients with Hodgkin's disease who were given TEM experienced definite subjective improvement within a few days. This

subjective improvement was manifested by a feeling of well-being, greater optimism, and increased appetite as soon as the transient nausea and anorexia abated. The duration of the subjective improvement was from a few days to three months.

Three patients who had not been treated with x-ray or HN2 previously had almost complete regression of peripheral lymph node enlargement. It is the authors' impression that the regression was somewhat slower than that observed following HN2. The diminution in size became apparent at three to seven days after completion of the course of TEM. With oral administration of TEM, regression usually commenced within a week. Although the data are insufficient for definite conclusions, it is the authors' impression that the remissions obtained in Hodgkin's disease with TEM were not as complete and did not last as long as those observed following

treatment with HN2 in single doses of 0.3 to 0.5 mg. per kilogram of body weight.²

Two patients had previously received x-ray therapy but no nitrogen mustard. In both, remissions of one month were obtained with TEM.

Four patients had previously received both x-ray and HN2. Of these, two patients (Cases 2 and 5) were still responding to HN2, and also responded to courses of TEM with satisfactory remissions of two and one months, respectively. The remaining three patients (Cases 5, 6 and 9) did not respond to HN2 therapy, and also had no objective improvement following TEM. It is concluded that little or no benefit is to be anticipated in Hodgkin's disease from the use of TEM if HN2 is ineffective. Probably the obverse is true also: One patient continued to have undulating fever despite repeated courses of TEM, and was given a course of HN2. No dif-

Chart 3.—Clinical and hematologic course of a patient with Hodgkin's disease (Case 1) receiving TEM and HN2. There was no effect upon the undulating fever, although the enlarged cervical lymph nodes were considerably reduced in size and there was subjective improvement.

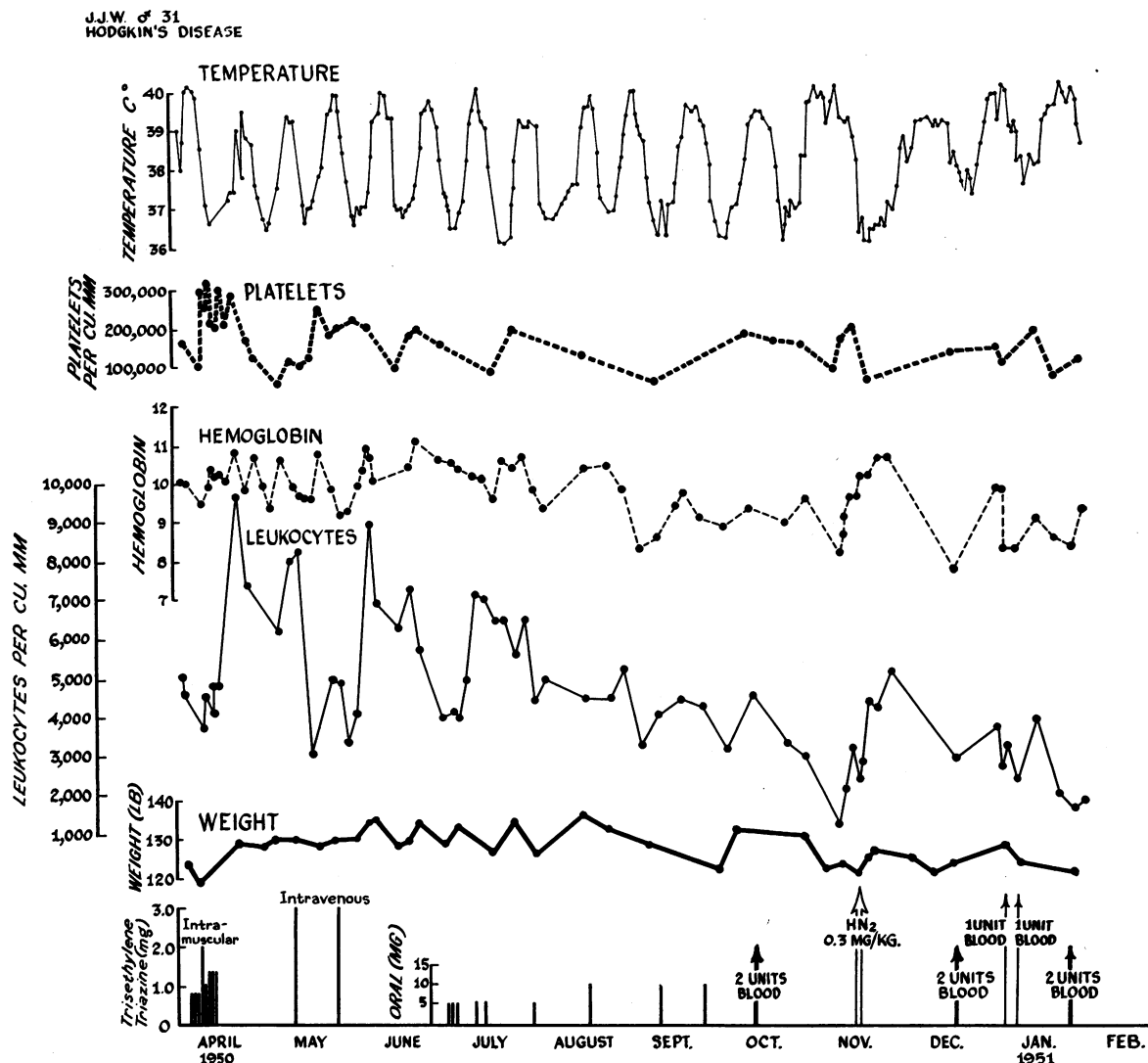


TABLE 1.—*TEM in the Treatment of Patients with Hodgkin's Disease and Other Lymphomas*

Case No.	Sex and Age	Duration of Disease (months)	Prior Therapy		General Condition	TEM Therapy			Effect	Length of Remission (months)	Outcome Following Last TEM Therapy	
			X-ray	HN2		Route*	Schedule† Admin./Days	Total Dose (mg.)				Dose (mg. per kg. of Body Weight)
HODGKIN'S DISEASE												
1.	M 31	54	—	—	Fair	IM	7/7	8.5	0.15	Good	1	Living—4 months
		55			Fair	IV	2/14	6	0.10	Good	1	
		57			Fair	O	9/90	60	Fair	2	
2.	M 23	60	+	+	Fair	IV	1/1	10	0.16	Good	2	Living—3 months
		62			Good	IV	1/1	10	0.16	Good	1	
3.	F 23	10	—	—	Good	O	2/2	20	0.4	Good	1	Living—2 months
		13			Good	O	4/4	20	0.4	Good	2	
		14			Good	O	4/4	20	0.4	Good	2	
		15			Good	O	2/8	10	0.2	Good	1	
4.	M 68	12	—	—	Good	IV	5/5	16.5	0.26	Good	3	Living—5 months
		15			Good	O	3/3	15	0.25	Good	1	
		16			Good	O	4/4	20	0.33	Good	1	
		17			Good	O	7/14	55	0.9	Toxic	..	
5.	M 33	36	+	+	Fair	IV	2/8	9	0.15	Fair	1	Died—1 month
		37			Fair	O	1/1	10	0.16	None	..	
		37.5			Poor	IV	1/1	10	0.16	Poor	..	
6.	F 29	17	+	+	Poor	IV	3/3	6	0.13	Fair	0.5	Died—1 month
		17.5			Poor	O	1/1	10	0.22	None	..	
7.	M 28	12	+	—	Fair	IV	3/3	7.5	0.15	Good	1.0	Died—2 months
		13			Fair	IV	3/3	7.5	0.15	Fair	0.5	
8.	M 26	4	—	—	Fair	IV	3/3	8.1	0.15	Good	0.5	Living—1 month
9.	F 27	24	+	+	Poor	IM	3/3	5.6	0.15	None	..	Died—1 month
LYMPHOSARCOMA												
10.	M 39	5	—	—	Fair	IM	7/7	7.0	0.10	Good	2	Died—1 month
		5.5			Fair	IM	5/5	10.0	0.14	Good		
		7.5			Fair	O	2/2	10.0	0.14	Fair		
11.	M 32	25	+	+	Poor	IM	7/7	4.9	0.10	Fair	0.5	Died—2 months
		26			Poor	IM	4/4	6.4	0.12	Poor	..	
12.	M 55	6	—	—	Good	O	1/1	5	0.08	Good	1	Living—1 month
		8			Good	IV	2/3	2	0.03	Good	1	
		8.5			Good	O	1/1	5	0.08	Fair	0.5	
		9.5			Good	O	1/1	5	0.08	Fair	1	
LYMPHOCYTIC LEUKEMIA												
13.	F 67	48	—	—	Good	IM	3/3	3.5	0.05	Good	3	Living—1 month
		55			Good	IM	2/3	1.5	0.02	Good	2	
		57			Good	O	1/1	5.0	0.07	Fair	1+	
14.	F 5	4	—	—	Fair	IM	1/1	0.5	0.03	Fair	1	Died—1 month
		5			Fair	O	1/1	2.0	0.13	Fair	0.5	
		6			Poor	O	1/1	2.0	0.15	Poor	..	
MYELOCYTIC LEUKEMIA												
15.	M 38	18	—	—	Fair	IM	5/5	5.4	0.1	None	..	Living—9 months
		18			Fair	IV	2/2	2.8	0.05	None	..	
16.	F 63	140	+	—	Fair	O	2/4	10	0.13	None	..	Living—5 months
		142			Fair	O	5/30	30	0.4	None	..	
MYCOSIS FUNGOIDES												
17.	M 58	170	+	+	Poor	IM	5/5	14.0	0.24	None	..	Died—1.5 months
18.	M 70	24	+	+	Fair	IM	7/7	6.8	0.10	None	..	Died—10 months
		26			Fair	IM	7/7	10.0	0.24	None	..	

* IM—Intramuscular; IV—Intravenous; O—Oral.

† The figures indicate the number of doses and the span of time over which they were given. Example: 7/7 means seven doses in seven days.

ference in effect in controlling the disease was noted (Chart 3).

Objective evidence of improvement following TEM therapy was judged by the effect upon lymph nodes, spleen, fever, and pruritus. Lymph node enlargement was reduced considerably in four patients, and moderately in two. It was not affected in two patients in whom previous HN2 therapy had also been ineffective, and one patient had no palpable lymph nodes. Splenomegaly was present in two patients. In one, the spleen was reduced in size, becoming no longer palpable. In the other patient, no longer responding to HN2, no effect was observed. Five of nine patients (Cases 1, 2, 3, 7 and 8) gained weight, up to 5 kg., during the month following TEM treatment. The one patient in good condition who did not gain weight (Case 4) received excessive doses which resulted in anorexia and pronounced bone marrow depression.

Three patients (Cases 1, 2 and 5) had fever as part of the course of the disease. TEM had no effect upon the febrile course in two patients, but did produce a remission of one month in the febrile course of one patient. Three patients had pruritus. In two, the pruritus was controlled, with complete relief for approximately two weeks. In one patient (Case 5), no longer responding favorably to either x-ray or HN2, the pruritus was not affected.

Lymphosarcoma and lymphocytic leukemia. Three patients with lymphosarcoma were treated with TEM. One patient (Case 11) in poor condition had slight reduction in the size of cervical, abdominal and thoracic masses, lasting for about two weeks, with subjective improvement and a weight gain of 2 kg. One patient (Case 10) with a rapidly progressing lymphoblastoma, had good remission for two months following two parenteral courses of TEM. The third course, given orally, produced

much less effect. The third patient (Case 12) had lymphosarcoma involving the cervical, axillary and inguinal lymph nodes. In this case the bone marrow cell count was 90 per cent lymphocytes. One oral administration of 5 mg. of TEM produced pronounced regression in lymph node enlargement and a decrease in the number of lymphocytes in the bone marrow to 20 per cent. Satisfactory improvement was achieved with a second intravenous course.

The hematologic course of one patient (Case 13) with chronic lymphocytic leukemia is recorded in Chart 4. There was a decrease in the number of leukocytes in the peripheral blood within a few days of therapy, followed by a slower reduction in cervical and axillary lymph node enlargement. The remission lasted for three months, and was again achieved by two subsequent courses of TEM.

Another patient (Case 14) had primitive cell leukemia, either lymphoblastic or monoblastic in type, with large submaxillary lymph nodes. Prompt decrease of the leukocyte count with disappearance of the immature cells occurred after one intramuscular administration of 0.5 mg. of TEM. The lymph nodes regressed to approximately two-thirds the original size during the next two weeks.

Other lymphomas. Two patients with myelocytic leukemia (Cases 15 and 16) were treated with TEM. No effects were observed on peripheral blood counts, on bone marrow, or on splenomegaly. There was no subjective improvement.

Two patients with generalized, advanced mycosis fungoides (Cases 17 and 18), who were no longer responding to the intravenous administration of HN2, were treated with TEM. Depression of the leukocyte and platelet counts, and temporary hypoplasia of the bone marrow were observed, without clinical improvement.

Other neoplastic diseases. As indicated in Table 2,

Chart 4.—Hematologic course of a patient with chronic lymphocytic leukemia (Case 13) treated with TEM. Associated with the decrease in the leukocyte count there was reduction in the size of cervical and axillary lymph nodes.

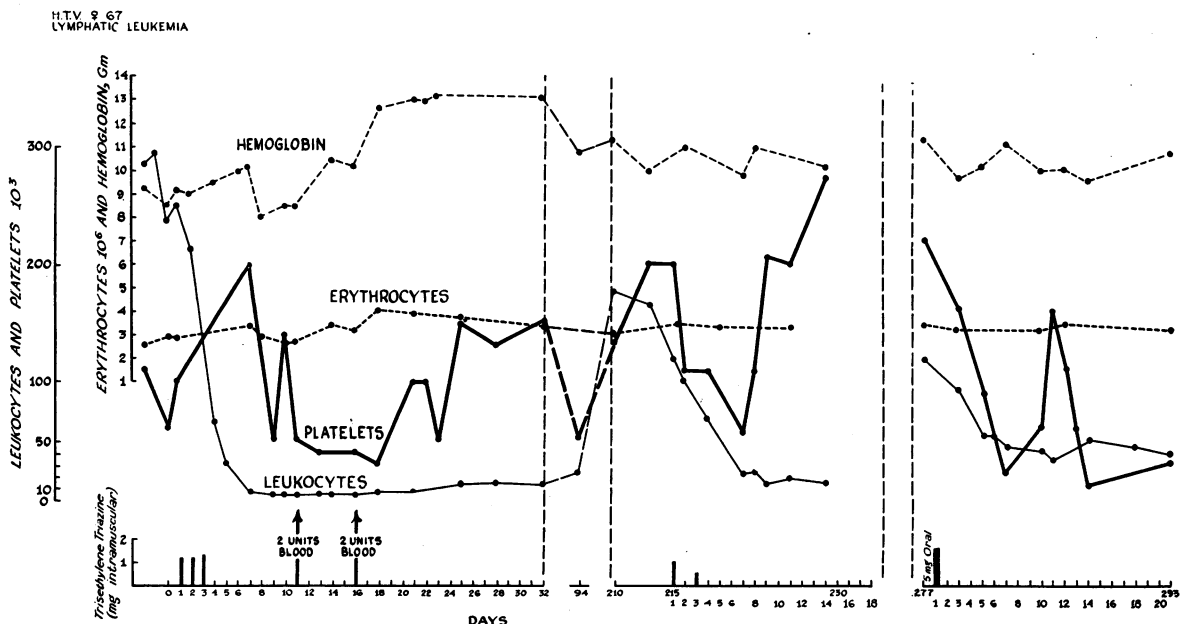


TABLE 2.—TEM in Patients with Neoplastic Diseases Other than Lymphomas.

Case	Sex and Age	Diagnosis	Route*	Days Treated	Total Dose	Dose (mg./kg.)	Effect on Tumor
19.	M 54	Carcinoma nasopharynx	IV	3	28	0.5†	None
20.	M 71	Carcinoma nasopharynx	O	3	15	0.28	None
			O	1	15	0.28	None
21.	M 42	Carcinoma lung	IV	5	12.5	0.28	None
22.	M 36	Carcinoma lung	IA	1	10.0	0.21	None
23.	M 28	Carcinoma testis	IM	7	4.9	0.10	Minimal‡
			IM	2	1.4	0.03	None
24.	M 28	Carcinoma testis	IV	1	9.0	0.15	None
25.	F 51	Carcinoma breast	IM	7	6.5	0.10	None
26.	M 46	Carcinoma kidney	IV	1	10.0	0.14	None
27.	F 38	Melanoma	IM	3	14.0	0.25	None
28.	F 48	Melanoma	IV	1	11.0	0.20	None
29.	M 18	Osteogenic sarcoma	IA	1	15.0	0.25	None
30.	F 2	Wilms's tumor	IA	1	2.6	0.20	Minimal‡
31.	M 5	Neuroblastoma	IA	1	4.0	0.25	None
32.	M 26	Thymoma	IV	3	9.0	0.15}	Minimal‡
			IV	2	6.0	0.1 }	

* IV—Intravenous; IM—Intramuscular; O—Oral; IA—Intra-arterial.

† This dose is fatal.

‡ Slight decrease in size of tumor masses for few days. All patients have died.

14 additional patients received treatment with TEM. No beneficial effects were observed. There was minimal but definite decrease in the size of the neoplastic mass in one patient with a teratocarcinoma of the testis, and in one child with a Wilms's tumor to whom TEM was administered through a catheter placed into the renal artery.¹ These effects lasted less than one week in each patient. In one patient with a thymoma which produced partial obstruction of the superior vena cava, treatment with TEM was followed by a reduction of the venous pressure from 28 to 12 cm. of water for approximately one week.

PATHOLOGY

Of the 32 patients in this series, nine of 18 patients with lymphomas, and all of 14 patients with other neoplasms have died. Necropsy was done in 21 of the 23 cases. The pathologic observations were similar to those in mice injected with TEM, and to those in patients following treatment with HN2.¹²

The changes in the tissues were dependent upon the dose of TEM administered and upon the time after the course of therapy. In general the cytotoxic effects were best noted in the bone marrow, the spleen, and to a lesser extent in lymph nodes.

No changes specific to the drug could be identified in the bone marrow earlier than one week following treatment with TEM. The most pronounced changes in the bone marrow were observed in cases in which autopsy was done between two and five weeks following the last course of therapy with TEM. The extent of the changes was in proportion to the dose of the agent. With doses of 0.25 to 0.5 mg. per kilogram of body weight there was complete disappearance of the cell elements. In the marrow the background was composed of remnants of supporting stroma, somewhat disrupted sinusoidal

walls and small islands of an eosinophilic-staining, mucinous-appearing material, probably extravasated serum. Occasional pyknotic nuclei and primitive cells were observed in this completely aplastic marrow (Figure 1). Approximately seven weeks following therapy with doses of 0.25 mg. per kilogram of body weight the marrow was hypercellular. There were no changes in bone trabeculae, and there was no increase of connective tissue in the marrow.

In the spleen the changes followed a somewhat similar pattern, the lymphocytes being specifically involved. With doses of TEM above 0.25 mg. per kilogram of body weight, the earliest changes were

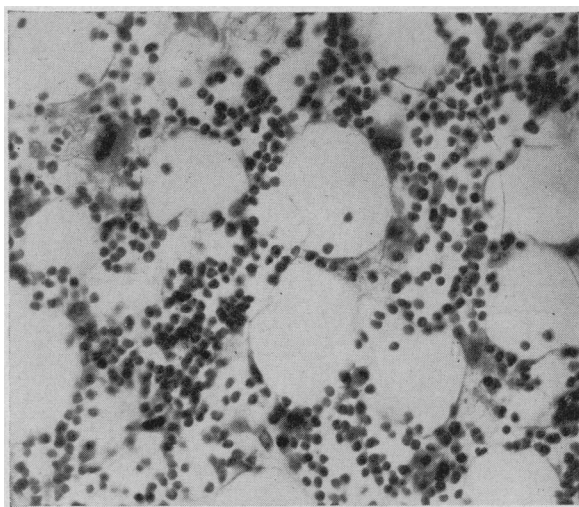


Figure 1.—Bone marrow of patient with epidermoid carcinoma of nasopharynx (Case 19), who died 11 days after receiving TEM, 0.5 mg. per kilogram of body weight, intravenously. Marrow elements are represented by a few large primitive cells in the stroma between fat cells; most of the remaining cells are erythrocytes. Hematoxylin- and eosin-stained. (X 320)

manifested by focal areas of necrotic tissue involving the tissue of a few Malpighian corpuscles (Figure 2). Within three weeks there was loss of lymphocytes from all of these areas, so that only the central arterioles remained. Considerable disruption of the red pulp, probably as part of the hemorrhagic diathesis, was also encountered. Later in the process the extravasated blood apparently degenerated and the pigment was contained within macrophages. Fibrous tissue proliferation occurred in the area of former lymphoid tissue. In other cases, in which smaller doses of TEM were given, no residual changes attributable to previous injury were observed in the spleen.

A few lymph nodes not replaced by tumor tissue were available for study. During the acute stages the

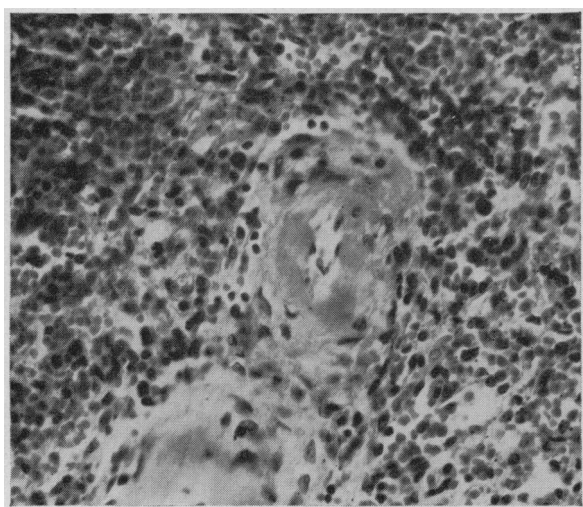


Figure 2.—Spleen of the patient in Case 19. Only a few lymphocytes remain of the lymphoid follicle and there is much extravasated blood in the pulp. Hematoxylin- and eosin-stained. (X 320)

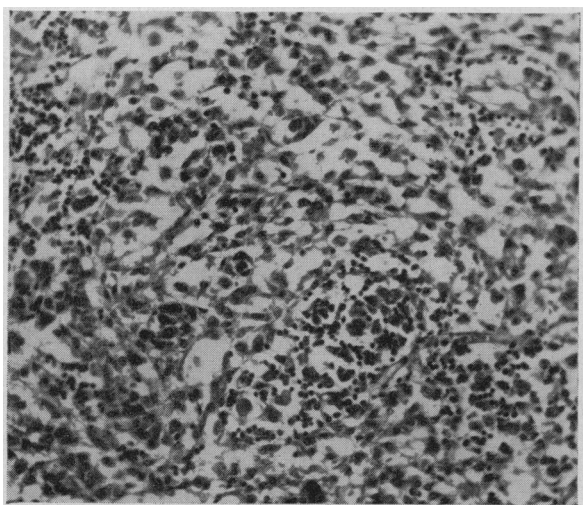


Figure 3.—Lymph node of patient with melanoma (Case 28) who died seven days following 0.2 mg. of TEM per kilogram of body weight, given intravenously. Lymphoid follicles are represented by a few groups of pyknotic lymphocytes and reticular cells form the bulk of the tissue. A group of melanoma cells appears in the lower center. Hematoxylin- and eosin-stained. (X 160)

lymphoid centers disappeared, the area consisting of the supporting stroma. Along with this there was considerable hyperplasia of the reticulum elements, many of these cells filling the sinusoids (Figure 3).

An interesting change was observed in one patient with Hodgkin's disease (Case 6) who died during the stage of bone marrow regeneration. In the submucosa of the small bowel were masses of plasma cells in areas usually containing many lymphocytes. No specific changes were observed in the gastrointestinal tract in other cases.

In the patient who received TEM in a dose of 0.5 mg. per kilogram of body weight (Case 19), in addition to the extreme effects upon the bone marrow and the spleen there were hemorrhages into the pulmonary alveoli and on serosal surfaces and mucous membranes. There was complete arrest of spermatogenesis in the testes.

In many cases necrosis of the tumor was a prominent feature. However, since this is also observed at autopsy in untreated cases, it could not be ascribed specifically to the drug. No cellular changes could be identified, and focal areas of the type described by Spitz¹² in tumors of the lymphoma group following therapy with nitrogen mustard, were not observed.

DISCUSSION

The clinical results of the present investigation of TEM are in agreement with the results reported by other groups.^{8, 13} Rhoads and co-workers⁸ studied 15 patients with Hodgkin's disease treated with TEM given orally. Fourteen of them had improvement. Two patients who had pruritus were relieved, and high fever was controlled for up to six weeks in two additional patients. The usual duration of improvement was six to 12 weeks. Objective improvement was also observed in three of four patients with myelocytic leukemia and in three of six patients with lymphocytic leukemia, but two patients with lymphosarcoma did not respond. One patient with mycosis fungoides and one with plasma cell myeloma also did not improve.

TEM appears to be a drug of some clinical usefulness in the same conditions in which nitrogen mustard is of some value: Hodgkin's disease, lymphosarcoma, lymphatic leukemia and perhaps other lymphomas. The remissions of the disease are of short duration, and no evidence is available that the life span is prolonged. The pharmacologic and toxicologic effects of TEM are in general the same as those of nitrogen mustard. The advantages of TEM as compared with HN2 are as follows: (a) TEM can be given intramuscularly and orally as well as intravenously, thus obviating the occasional complication of phlebotrombosis produced by nitrogen mustard. The oral route of administration also allows more continual and more regularly spaced treatments than are perhaps possible with nitrogen mustard. (b) TEM produces much less nausea and vomiting than does nitrogen mustard. Headache and diarrhea are also reduced. Two patients of the present series refused any further therapy with HN2 because of

severe reactions of vomiting and headache, but were satisfactorily continued on TEM.

There are also definite disadvantages of TEM as compared with HN2: (a) TEM has a narrower chemotherapeutic range than nitrogen mustard. With TEM given parenterally, 0.5 mg. per kilogram of body weight is fatal, and a single course should not exceed 0.25 mg. per kilogram of body weight. With oral administration, 0.4 mg. per kilogram of body weight, given in daily doses of 5 to 10 mg., appears to be the upper level of safe dosage for a single course of approximately one week. (b) Lymphocytic leukemia appears to be extremely sensitive to TEM. The first course, given intravenously or intramuscularly, should not exceed 0.05 mg. per kilogram of body weight, and oral dosage should not exceed 0.1 mg. per kilogram of body weight. This caution is applicable not only to lymphocytic leukemia with frank leukemic blood picture but also to subleukemic lymphocytic leukemia and to lymphosarcoma with bone marrow involvement. (c) The ease of administration of TEM, particularly orally, and the minimal immediate reactions are hazards as well as advantages. The severe effects on the bone marrow and the narrow chemotherapeutic range make imperative the closest clinical and hematological observation for at least three weeks following the termination of the therapy.

REFERENCES

1. Bierman, H. R., Miller, E. R., Byron, R. L., Dod, K. S., Kelly, K. H., and Black, D. H.: Intra-arterial catheterization of viscera in man, *Am. J. Roentgenology* (in press).
2. Bierman, H. R., Shimkin, M. B., Mettler, S. R., Weaver, J., Berry, W. C., and Wise, S. P.: Methyl-bis (beta-chloroethyl) amine in large doses in the treatment of neoplastic diseases, *Calif. Med.*, 71:117-125, 1949.
3. Burchenal, J. H., Crossley, M. L., Stock, C. C., and Rhoads, C. P.: The action of certain ethylenimine (aziridine) derivatives on mouse leukemia, *Arch. Biochem.*, 26: 321-323, 1950.
4. Gilman, A., and Philips, F. S.: The biological actions and therapeutic applications of the B-chloroethyl amines and sulfides, *Science*, 103:409-415, 1945.
5. Heston, W. E.: Induction of pulmonary tumors in strain A mice with methyl-bis (B-chloroethyl) amine hydrochloride, *J. Natl. Cancer Inst.*, 10:125-130, 1950.
6. Lewis, M. R., and Crossley, M. L.: Retardation of tumor growth in mice by oral administration of ethylenimine derivatives, *Arch. Biochem.*, 26:319-320, 1950.
7. Matthews, W. B.: A trial of B-naphthylde-2-chloroethylamine (R48) in leukemia, Hodgkin's disease, and allied diseases, *Lancet*, 1:896-899, 1950.
8. Rhoads, C. P., Karnofsky, D. A., Burchenal, J. H., and Craver, L. F.: Triethylene melamine in the treatment of Hodgkin's disease and allied neoplasms, *Trans. Assn. Amer. Phys.*, 63:136-146, 1950.
9. Rose, F. L., Hendry, J. A., and Walpole, A. L.: New cytotoxic agents with tumor-inhibitory activity, *Nature*, 165: 993-996, 1950.
10. Shimkin, M. B.: Induced pulmonary tumors in mice. II. Reaction of lungs of strain A mice to carcinogenic hydrocarbons, *Arch. Path.*, 29:239-255, 1940.
11. Shimkin, M. B.: Neoplastic diseases, *Ann. Rev. Med.*, 1:179-198, 1950.
12. Spitz, S.: The histological effects of nitrogen mustards on human tumors and tissues, *Cancer*, 1:383-398, 1948.
13. Wright, L. T., Wright, J. C., Prigot, A., and Weintraub, S.: Remissions caused by tri-ethylene melamine in certain neoplastic diseases, *J. Natl. Med. Assn.*, 42:343-351, 1950.

ADDENDUM

Karnofsky, D. A., Burchenal, J. H., Armistead, O. C., Southam, C. M., Bernstein, J. L., Craver, L. F., and Rhoads, C. P.: Triethylene melamine in the treatment of neoplastic disease, *A.M.A. Arch. Int. Med.*, 87:477-516, 1951.